Environmental Health Perspectives Supplements
Vol. 101 (Suppl. 3): 309–315 (1993)

Nitrated Polycyclic Aromatic Hydrocarbons: A Risk Assessment for the Urban Citizen

by Lennart Möller,[1] Ingemar Lax,[2] and Lennart C. Eriksson[3]

Nitrated polycyclic aromatic hydrocarbons (nitro-PAHs) are formed during incomplete combustion. Sources include emissions from vehicles (mainly diesel vehicles), heating, smoking, certain types of food processing, and incomplete combustion in general. Nitro-PAHs are direct-acting mutagens, and a number of them have been shown to be carcinogens. 2-Nitrofluorene (NF) represents a model substance for the nitro-PAHs. An attempt has been made to calculate the human cancer risk due to exposure to nitro-PAHs by two different models. In the first model, genotoxic lesions were transferred to units of Gray (γ-irradiation), and in the second model a mega study (24,000 animals) on the carcinogenicity of one metabolite of NF was used to elucidate the risk. Gamma-irradiation of the rat liver gave rise to preneoplastic foci in a dose-dependent manner. which was statistically significant. The Gray-equivalents of chemically (NF) induced foci were calculated, and from the human nitro-PAH exposure, expressed in Sievert, a human risk estimate was calculated. In the second model, an extrapolation from laboratory animals to man was performed because tumor data on 2-acetylaminofluorene (AAF), a major metabolite of NF, were available in the literature. The tumor dose–response data on AAF was linear for tested lifetime doses. The results of both models agreed, with a risk range of 0.15–49 $\times 10^{-6}$ on human cancer risk for an urban citizen.

Introduction

Incomplete combustion is a major problem in terms of pollution. Examples include emissions from energy production, industrial processes, vehicles, and smoking. The biological effects of incomplete combustion can be divided into effects on human health or on the ecosystem. Both effects can be acute or long term. The different biological responses can be related to each other because the same substance in the emissions can give rise to several reactions in the organism or the ecosystem. One such example is the group of nitrated polycyclic aromatic hydrocarbons (nitro-PAHs). For the formation of nitro-PAHs, incomplete combusted organic material (PAH) and oxidized nitrogen (NO_x) are necessary. A low pH (SO_2, NO_x) catalyzes the reaction. NO_x is one important combustion product responsible for acidification of the environment, acute health effects (*1*), as well as formation of nitro-PAHs (*2*), which are strong genotoxic agents in mammalian systems (*3–7*). Because the formation of nitro-PAHs is catalyzed by a low pH, the reaction product, NO_x, catalyzes its own reaction with PAHs in the formation of nitro-PAHs.

Nitro-PAHs are found in emissions from diesel- (*8*) and gasoline-powered (*9*) vehicles, in the exhaust from kerosene heaters (*10*), in urban air (*11–14*), in river sediments (*15*), and in certain food products (*16,17*). Nitro-PAHs can be formed during combustion or as a result of photochemical reactions of PAHs (*18*) or amino-PAHs (*19*). Nitro-PAH formation has also been reported to occur in the water phase with nitrite as a donor of the nitro group (*20,21*).

Nitro-PAHs are a group of at least 200 different substances. Many of them are mutagens (*22–25*), and the most potent bacterial mutagens known today, the dinitropyrenes, are found in this group (*26*). A number of the nitro-PAHs are also carcinogenic in laboratory animals (*27,28*).

2-Nitrofluorene (NF) is one of the more common nitro-PAHs, found in the environment (*29–33*) with 1-nitropyrene (NP). NF has been suggested to be a model substance for nitro-PAHs in the gas and particle phase, whereas NP is regarded to be a model substance for nitro-PAHs in the particle phase (*34*).

NF is a mutagen (*25*) as well as a carcinogen (*28*) in laboratory animals. NF was elucidated by the International Agency for Research on Cancer (IARC), and the conclusion was that there is "sufficient evidence" for the carcinogenicity in experimental animals. Concerning the evaluation for human health the statement was that NF is a "possible carcinogen to humans" (*35*). The evaluation for diesel exhaust was that it is "probably carcinogenic" to humans (*35*).

[1]Center for Nutrition and Toxicology, Karolinska Institute, Novum Research Park, S-141 57, Huddinge, Stockholm, Sweden.
[2]Department of Hospital Physics, Karolinska Hospital, Box 60500, S-104 01, Stockholm, Sweden.
[3]Department of Pathology, Karolinska Institute, Huddinge University Hospital, S-141 86, Huddinge, Stockholm, Sweden.
Address reprint requests to L. Möller, Center for Nutrition and Toxicology, Karolinska Institute, Novum Research Park, S-141 57, Huddinge, Stockholm, Sweden.

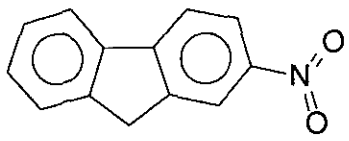

FIGURE 1. The chemical structure of 2-nitrofluorene.

NF has been studied in detail in our laboratory from an analytical point of view (*36*), and with regard to metabolism (*37–39*), lung effects (*40,41*), and genotoxic effects (*42–44*). A review of NF's prevalence and biological effects has also been published (*45*). The chemical structure of NF is shown in Figure 1.

Results and Discussion

For the group of nitro-PAHs, epidemiology or studies on specially selected groups are more or less impossible to perform because nitro-PAHs constitute only part of the products of incomplete combustion. It is extremely hard to define a group of people that is exposed to nitro-PAHs and a control group that is exactly the same from all aspects except that it is not exposed to nitro-PAHs. Nitro-PAHs always occur with other carcinogens (combustion products), and therefore another approach must be used to elucidate the genotoxic risk.

2-Nitrofluorene, a Potent Genotoxic Model Compound for Nitro-PAHs

2-Nitrofluorene (NF) and 1-nitropyrene (NP) have been selected as model compounds for the group of nitro-PAHs. The basic structures, fluorene and pyrene, are common in emissions from incomplete combustion. Because nitrogen oxides are also present during combustion, the reaction products NF and NP are common in environmental analyses (*8–17*). NP occurs in the particle phase, whereas NF is found with a 50/50 distribution in the particle and semivolatile phase (*34*).

In our studies, NF was selected as the model compound. NF is a direct-acting mutagen in bacterial test systems and is often used as a positive control in genotoxic assays. In Table 1, a summary of some of the existing data on the genotoxicity of NF is shown.

Table 1. A summary of the biological effects of 2-nitrofluorene.

Assay	Effect[a]	Reference
Sister chromatid exchange	+	(*3,5*)
Initiator	+	(*44*)
Promoter	+	(*44*)
Carcinogenicity	+	(*27,56*)
Formation of DNA adducts	+	(*69*)
Micronuclei assays	−	(*6,70–72*)
Bacterial mutagenicity (Salmonella)	+	(*74–76*)
Bacterial mutagenicity (*E. coli*)	+	(*77*)
Mutagenicity, mouse lymphoma assay	+	(*3,73,78*)

[a](+) Positive effect; (−) no effect.

Metabolism of 2-Nitrofluorene

Although NF is a chemically stable molecule, it is extensively metabolized in the organism. After oral administration of NF, the major part of the dose is excreted within 48 hr (*37,39*). After 4 hr, approximately 2% of the dose has been metabolized by the intestinal microflora, absorbed, and metabolized (several steps) in the liver, distributed in the circulation, filtered by the kidneys, and excreted in urine. The excretion of metabolites is accompanied by excretion of mutagenicity. Typically, direct-acting mutagenicity (-S9) dominates over mutagenicity in the prescence of S9, both in urine and feces (*37,39*).

The *in vivo* formation (*37*) of the potent carcinogen 2-acetylaminofluorene [AAF (*46*)] is indicated. After an oral dose of NF to rats, NF is reduced to 2-aminofluorene (AF) by the intestinal microflora, acetylated to AAF, and further hydroxylated in the liver, resulting in OH-AAF compounds, which can be excreted as such or in conjugated form. This metabolic route is quantitatively the most important. AAF has been a model compound for chemical carcinogenesis since the Wilson's early finding of its carcinogenic potential in 1941 (*47*). AAF has been used in a number of bioassays and has been characterized from many different points of view (*48*). AAF is not found in the environment, and occupational exposure can only occur when AAF is used in research. It is thus of concern when an environmental pollutant (NF) commonly found in diesel exhaust (*9,49–51*) has the capacity to be metabolized to this potent carcinogen *in vivo*. Other nitro-PAHs have been shown to form acetylated metabolites, although the biological significance of these metabolites is not known (*52,53*).

After oral administration of NF, an alternative metabolic route results in the formation of OH-NFs. This is seen after induction of the cytochrome P450 system *in vivo*. It is also seen in isolated, perfused lung and liver as well as in germ-free animals (*37–39*). OH-AAFs are considered to be detoxification products (*54*) and have a low mutagenic potency (*55*). OH-NFs, on the other hand, are more mutagenic than NF itself (*39*). So far, nothing is known about the carcinogenic potential of OH-NFs, but it cannot be excluded that they are carcinogenic. They may, for example, be involved in the tumor formation seen in the forestomach after oral dosing of NF. No forestomach tumors are seen after administration of AF or AAF. OH-NFs may also play a role in the formation of subcutaneous tumors after skin application of NF (*28,56*). The metabolism of NF is shown in Figure 2.

Are Animal Data Relevant to Humans?

One can always argue whether data on animal metabolism are relevant to humans, but in the case of nitro-PAHs, there are a number of factors indicating that animal studies are relevant to the human situation: *a*) Reduction of nitro-PAHs to amino-PAHs can be performed by anaerobic fecal bacterial suspensions from humans as well as from rats (*57–59*). *b*) Human liver S9 bioactivates AF and AAF to mutagens (*60*). *c*) Human hepatoma cell lines can

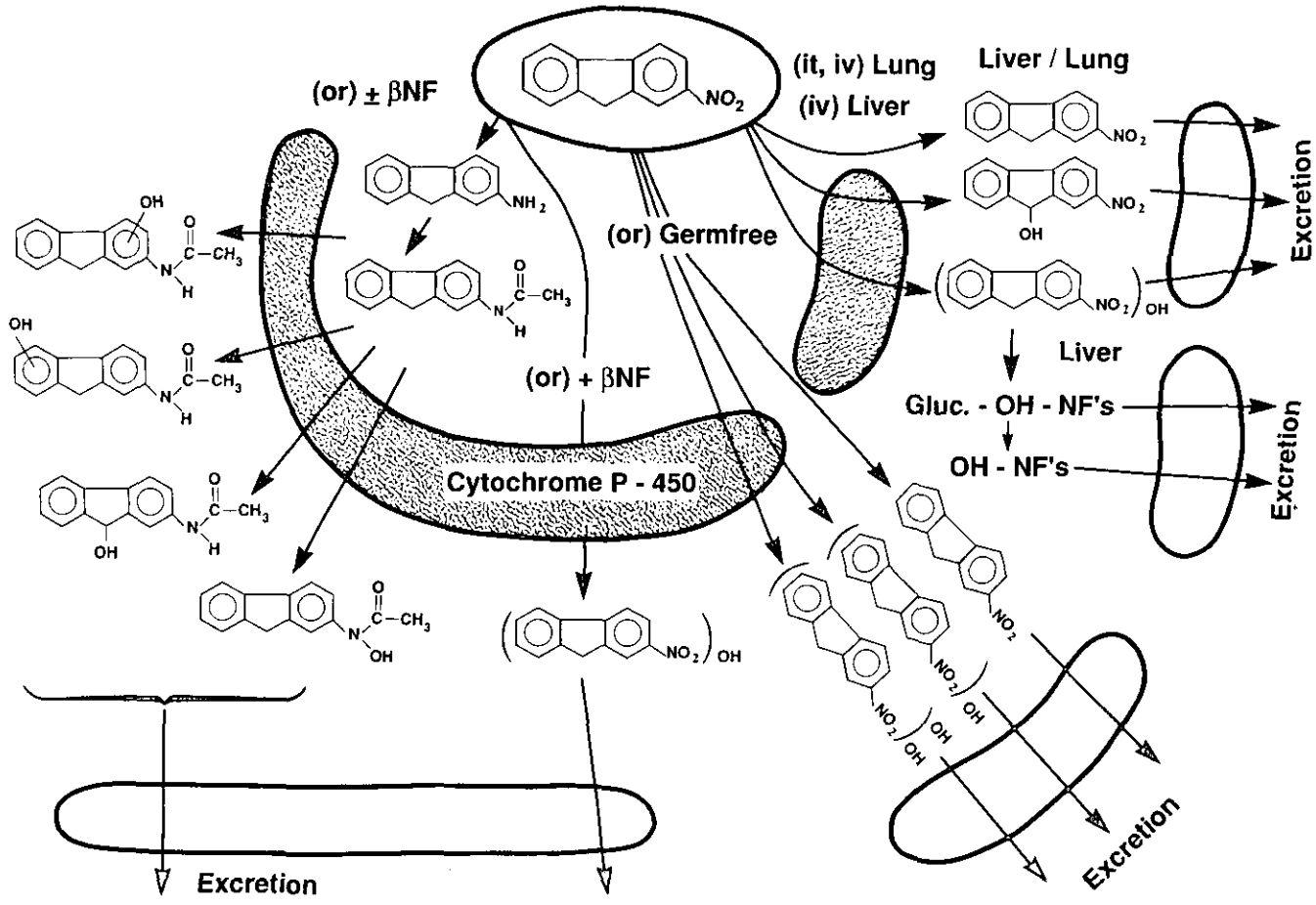

FIGURE 2. The pathways for the metabolism of 2-nitrofluorene.

perform nitroreduction as well as ring hydroxylation of NP (*61*). *d*) Liver microsomal metabolism of AAF is similar in rats and humans (*62*). *e*) Human lymphocytes metabolize AAF to ring- and N-OH derivatives of AAF (*63*). *f*) AAF metabolism is similar in cultures of epithelial cells from human and rat bladder (*64*). *g*) It has been shown that the carcinogen AAF given orally to humans results in the same urinary metabolites as in the rat (*65*).

Risk Calculations Using AAF

In the AAF model, combined data have been used from the metabolic pathways (NF metabolized to AAF *in vivo*) and a mega-study (*66*) performed on 24,000 animals to elucidate the carcinogenic potential of AAF at low lifetime dosing. The tumor data on the liver have been used for the extrapolation to man. The dose–response curve for tumor formation in the animal study after lifetime dosing was linear. In the calculations (*67*), the animal data have been extrapolated to man. The positive and negative comments that could be made regarding this model are found in Table 2. With an estimated human lifetime dose range for an urban citizen, as discussed by Möller et al. (*73*), the human cancer risk is $p(x) = 1.1 - 49 \times 10^{-6}$. Weaknesses and strengths of the AAF model are discussed in Table 2.

Table 2. Weaknesses and strengths of the 2-acetylaminofluorene (AAF) model.

Weaknesses
Data extrapolated from animals to man.
A part of the human dose of 2-nitrofluorene (NF) might undergo ring-hydroxylation instead of reduction and acetylation. However, much more potent mutagens (carcinogens?) are formed via that route.
Strengths
Many animals in the cancer study (24,000).
Known metabolism for the model compound (NF).
Low lifetime doses of AAF gives rise to tumors.
The dose–response curve was linear for liver tumors on which the calculations were performed.
The metabolism in laboratory animals and man is probably similar.
The AAF model is an *in vivo* model.
AAF is considered a human carcinogen.
Enzymes necessary for the metabolic pathways are found in man.

Risk Calculations Using γ-Radiation Eqivalents

The γ-model is totally different from the AAF model in that known human risk data on γ-irradiation is the basis for the risk calculation. The genotoxic effects in the rat liver after exposure to NF occur as preneoplastic lesions (*44*). The same genotoxic lesions can be formed after exposure to γ-irradiation of the rat livers (*67*). The dose of γ-irradiation was exact in terms of dose maxima, exposed area, standard deviation of the dose, etc., because γ-irradiation apparatus for human cancer treatment was used (*67*). In this way the genotoxic lesions caused by NF could be converted to γ-irradiation equivalents (Gy). The total human dose of nitro-PAHs could then be expressed in radiation units, which made it possible to use human risk data on carcinogenesis after γ-irradiation exposure to large populations (*68*). With the same estimated life dose of nitro-PAHs as used in the AAF model, the human cancer risk for an urban citizen is $p(x) = 0.30 - 39 \times 10^{-6}$. Weaknesses and strengths of the γ-model are discussed in Table 3.

Human Dose of Nitro-PAHs

The basis for the risk calculations is the human lifetime dose of nitro-PAHs. Based on literature data, an estimated lifetime dose range has been calculated, which is discussed in detail in Möller et al. (*67*). The combined risk range is $0.15–49 \times 10^{-6}$. In the dose calculations, food has been considered to be zero in terms of nitro-PAH dose, although this is not the case; food can even be the predominate source of nitro-PAHs (*16,17*). Cities like Berlin and Beijing represent the upper risk range, and cities like Tokyo and Kawasaki represent the lower risk range. High traffic intensity, a lot of diesel vehicles, and coal as source of energy lead to doses that pose a high risk. Some comments on the dose are shown in Table 4. In Tables 5 and 6, NF analyses in urban air and particle extracts are shown. In Table 7 the factors that could increase or decrease the calculated risk for nitro-PAHs are discussed. Although no definite risk limit has been set, a risk of 1×10^{-6} is in general is considered to be the limit for an unacceptable risk. That risk limit could be exeeded by a factor of up to 50 times in the case of nitro-PAHs.

Table 3. Weaknesses and strengths of the γ model.

Weaknesses
Are foci generated from chemicals and γ-irradiation in the same manner?
Recent human risk data on γ-irradiation indicate that the risk is underestimated.
Strengths
Well-known risk data on humans after exposure to γ-irradiation exists.
Conversion of genotoxic lesions to radiation-equivalents.
The γ-model is an *in vivo* model.

Table 4. Weaknesses and strengths regarding current knowledge on the human exposure to nitro-PAHs.

Weaknesses
Are the literature data on nitro-PAH levels representative?
Sampling temperatures are not given (might influence distribution gas/particle phase).
Limited information available on the food levels of nitro-PAHs. Could be a major source of nitro-PAHs.
Can plants (for food consumption) along roads absorb and metabolize nitro-PAHs?
What is the relationship between oral and inhaled dose?
Are the potent direct-acting mutagens, the OH-NFs, also carcinogens?
Strengths
A large number of analyses on urban air.
2-Nitrofluorene seems to be a good model for nitro-PAHs in terms of dose.
An equal distribution of nitro-PAHs in the urban environment due to a very large number of sources.

Abbreviations: nitro-PAHs, nitrated polycyclic aromatic hydrocarbons; OH-NF,

Table 5. Quantitation of 2-nitrofluorene (NF) in different environments.

Environment	NF, pg/m³	Comment	Reference
Tokyo[a]	24	Winter, residential area	(*79*)
	50	Summer, residential area	(*79*)
Kawasaki[a]	71	Autumn, industrial area	(*79*)
Beijing[b]	190	Residential area, close to road	(*79*)
	290	Residential area, close to road	(*79*)
	36	Background level	(*79*)
	79	Background level	(*79*)
	700	Maximum level	(*79*)
Berlin[c]	1780	$n = 8$	(*14*)
	1510	$n = 11$, over a year	(*14*)
	1880	Energy production dominates	(*80*)
	1540	Traffic dominates	(*80*)
Japan	1.5 μg/kg	River sediment	(*15*)
Kerosene heater	568 ng/m³	In the exhaust	(*10*)
	19.8 ng/m³	Close to kerosene heater	(*10*)

[a]Big cities, relatively few vehicles, catalytic cleaning devices, main traffic flow by underground, and cities located on the coast.
[b]Big city, very few vehicles, diesel vehicles, no catalytic cleaning of vehicle emissions, and extensive heating/cooking with coal.
[c]Big city, many vehicles, typical western-type city, although former East Berlin has coal as a fuel for heating, which might influence the air quality in former West Berlin where the analyses have been performed.

Table 6. Quantitative data on 2-nitrofluorene (NF) in vehicle emissions.

Source	NF Level	Reference
Diesel exhaust, bus	0.13–1.5 μg/km	(*50*)
HDD, 100% load, moderate speed	0.63 μg/g (soot)	(*49*)
HDD, 75% load, high speed	8.8 μg/g	(*49*)
Diesel muffler	52.2 μg/g	(*9*)
SRM 1650[a]	15 μg/g	(*49*)
HDD, idle	84 μg/g	(*51*)
HDD, zero-load, high speed	62 μg/g	(*51*)
HDD, full-load, high speed	1.9 μg/g	(*51*)
LDD, gas phase	90 μg/mile	(*34*)
LDD, particle phase	97 μg/mile	(*34*)
Gasoline muffler	0.16 μg/g	(*9*)

Abbreviations: HDD and LDD, heavy and light duty diesels, respectively.
[a]The National Bureau of Standards Reference Diesel Particulate, USA.

Table 7. Summary of factors that can increase or decrease the risk of nitro-PAHs.

Increase risk

- Synergism with other air pollutants occurring in vehicle emissions, urban air, or in cigarette smoke (nitro-PAHs always occur together with PAHs).
- Nitro-PAH emmission from kerosene heaters (*10*).
- Certain combustions may generate more nitro-PAHs than vehicles [airplanes and ships (*51,74*)].
- Cigarette smoke might be an unknown source for nitro-PAHs.
- Water-phase reactions (PAH + nitrite) could be an additional source for nitro-PAHs (*20,21*).
- Cooking could be a predominate source of nitro-PAHs if the Japanese data on chicken are generally applicable (*17*).
- Nitro-PAHs can act as tumor promoters (*44*) and/or affect tumor progression.
- Exposure to nitro-PAHs could lead to an increased risk of miscarriages and malformed offspring because metabolites of 2-nitrofluorene (NF) can cause malformation in laboratory animals (*82*).
- Certain other substances can function as co-carcinogens with nitro-PAHs: one example is benzo(*a*)pyrene (BaP) (*83*).
- Compared to other nitro-PAHs, NF, which was the base for calculations, has a low bacterial mutagenicity.
- Contamination of the environment of nitro-PAHs might be an addition to the lifetime dose (*15*).
- If particles are very small (<1.5 μm), they contain more nitro-PAHs (*84*).
- Certain risk behaviors (being a child and breathing closer to the sources of emissions for instance) are not included.
- Certain risk situations are not included (indoor exposure of diesel exhaust in garages, mines, ferries, etc.).
- Certain occupational risk behavior is not included such as cutaneous exposure of soot (workshops, chimney sweeps, etc.).
- Metabolism of other nitro-PAHs might be more important than NF in terms of genotoxicity.
- Metabolism without intestinal microflora (oxidative pathway) leads to the formation of more genotoxic metabolites than 2-acetylaminofluorene (AAF) (*37–39*).
- Local sources in the countryside could give rise to relatively high levels of nitro-PAHs (diesel tractors, for instance).
- The risk calculations performed on the victims from Hiroshima and Nagasaki, which is the basis for the rad equivalent calculation, can be underestimated (*81*).
- If nitro-PAHs reacts with cell components other than DNA, it is possible that other toxic reactions might occur (i.e., reactions with proteins).

Decrease risk

- Measured levels of NF in Berlin might not be representative of cities in general (it has been assumed in the calculation that urban levels can be as low as 10% of the Berlin concentration, which is one reason for the width of the risk range).
- If NF is not a good model for nitro-PAHs in general in terms of biological effects.
- The increased use of catalytic cleaning devices, although this is not the case for diesel vehicles for the moment (which is so far the main known source for nitro-PAHs).
- If animal models are not relevant to human risk.
- Metabolism of other nitro-PAHs is less important than NF in terms of genotoxic risk.
- If metabolism of NF via the oxidative pathway leads to less genotoxic metabolites than AAF.
- If γ-generated foci are different from chemically induced foci.
- If antagonism between NF and other xenobiotics reduce the promotive response.

REFERENCES

1. Lindvall, T. Health effects of nitrogen dioxide and oxidants. Scand. J. Work Environ. Health. 11: 10–28 (1985).
2. Tokiwa, H., Nakagawa, R., Morita, K., and Ohnishi, Y. Mutagenicity of nitro derivatives induced by exposure of aromatic compounds to nitrogen dioxide. Mutat. Res. 85: 195–205 (1981).
3. McCoy, E. Role of metabolism on the mutagenicity of nitroarenes. In: Biochemical Basis of Chemical Carcinogenesis (H. Greim, M. Jung, M. Kramer, H. Marquardt, and F. Oesch, Eds.), Raven Press, New York, 1984, p. 57.
4. Danford, N., Wilcox, P., and Parry, J. M. The clastogenic activity of dinitro-pyrenes in a rat liver epithelial cell line. Mutat. Res. 105: 349–355 (1982).
5. Nachtman, J. P., and Wolff, S. Activity of nitro-polynuclear aromatic hydrocarbons in the sister chromatid exchange assay with and without metabolic activation. Environ. Mutagen. 4: 1–5 (1982).
6. Neal, S. B., and Probst, G. S. Chemically-induced sister chromatid exchange in vivo in bone marrow of Chinese hamsters, an evaluation of 24 compounds. Mutat. Res. 113: 33–43 (1983).
7. Tucker, J. D., and Ong, T. Induction of sister chromatid exchanges and chromosome aberrations in human peripheral lymphocytes by 2,4,7-rinitro 9-fluorenone. Mutat. Res. 138: 181–184 (1984).
8. Hartong, A., Kraft, J., Schulze, J., Kiess, H., and Lies, K.-H. The identification of nitrated polycyclic aromatic hydrocarbons in diesel particulate extracts and their potential formation as artifacts during particulate collection. Chromatographia 19: 269–273 (1984).
9. Handa, T., Yamauchi, T., Ohnishi, M., Hisematsu, Y., and Ishii, T. Detection and average content levels of carcinogenic and mutagenic compounds from the particulates on diesel and gasoline engine mufflers. Environ. Int. 9: 335–341 (1983).
10. Tokiwa, H., Nakagawa, R., and Horikowa, K. Mutagenic/carcinogenic agents in indoor pollutants: the dinitropyrenes generated by kerosene heaters and fuel gas and liquified petroleum gas burners. Mutat. Res. 157: 39–47 (1985).
11. Ramdahl, T., Becher, G., and Björseth, A. Nitrated polycyclic aromatic hydrocarbons in urban air particles. Environ. Sci. Technol. 16: 861–865 (1982).
12. Gorse, R. A., Riley, T. L., Ferris, F. C., Pero, A. M., and Skewes, L. M. 1-Nitropyrene concentration and bacterial mutagenicity in on-road vehicle emissions. Environ. Sci. Technol. 27: 198–202 (1983).
13. Tokiwa, H., Kitamori, S., Nakagawa, R., and Ohnishi, Y. Mutagens in airborne particulate pollutants and nitro derivatives produced by exposure of aromatic compounds to gaseous pollutants. Environ. Sci. Res. 27: 555–567 (1983).
14. Moriske, H.-J. Polare verbindungen im stadtaerosol. VDI Fortschrittberichte, Reike 15, No. 42, VDI-Verlag, Düsseldorf, 1986.
15. Sato, T., Kato, K., Ose, Y., Nagase, H., and Ishikawa, T. Nitroarenes in Suimon river sediment. Mutat. Res. 157: 135–143 (1985).
16. Ohnishi, Y., Kinouchi, T., Manabe, Y., Tsushi, H., Otsuka, H., Tokiwa, H., and Otofujii, T. Nitro compounds in environmental mixtures and foods. In: Short-Term Bioassays in the Analysis of Complex Environmental Mixtures, IV (M. D. Waters, Ed), Plenum Press, New York, 1985, pp. 195–204.
17. Kinouchi, T., Hideshi, T., and Ohnishi, Y. Detection of 1-nitropyrene in Yakatori (grilled chicken). Mutat. Res. 171: 105–113 (1986).
18. Tokiwa, H., Nakagawa, R., Morita, K., and Ohnishi, Y. Mutagenicity of nitro derivatives induced by exposure of aromatic compounds to nitrogen dioxide. Mutat. Res. 85: 195–205 (1986).
19. Okinaka, R. T., Nichols, J. W., Whaley, T. W., and Strniste, G. F. Phototransformation of 2-aminofluorene into N-oxidized mutagens. Carcinogenesis 5: 1741–1743 (1984).
20. Ohe, T. Mutagenicity of photochemical reaction products of polycyclic aromatic hydrocarbons with nitrite. Sci. Total Environ. 39: 161–175 (1984).
21. Suzuki, J., Hagino, T., and Suzuki, S. Formation of 1-nitropyrene by photolysis of pyrene in water containing nitrite ion. Chemosphere 16: 859–864 (1987).
22. Rosenkranz, H. S., and Mermelstein, R. M. The genotoxicity, metabolism and carcinogenicity of nitrated polycyclic hydrocarbons. J. Environ. Sci. Health 2: 221–272 (1985).
23. Tokiwa, H., Nakagawa, R., and Ohnishi, Y. Mutagenic assay of aromatic nitro compounds with Salmonella typhimurium. Mutat. Res. 91: 321–325 (1981).
24. Pitts, J. N., Jr., Harger, W., Lokensgaard, D. M., Fitz, D. R., Scorziell, G. M., and Mejia, V. Diurnal variations in the mutagenicity of airborne particulate organic matter in California's south coast air basin. Mutat. Res. 104: 35–41 (1982).

25. Wang, Y. I. Y., Rappaport, S. M., Sawyer, R. F., Talcott, R. E., and Wei, E. T. Direct-acting mutagens in automobile exhaust. Cancer Lett. 5: 39–47 (1987).
26. Pederson, T. C., and Siak, J.-S. The role of nitroaromatic compounds in the direct acting mutagenicity of diesel particle extracts. J. Appl. Toxicol. 1: 54–60 (1981).
27. El-Bayoumy, K., Hecht, S., and Hoffman, D. Comparative tumor initiating activity on mouse skin of 6-nitrobenzo(a)pyrene, 6-nitrochrysene, 3-nitroperylene, 1-nitropyrene and their hydrocarbons. Cancer Lett. 16: 333–337 (1982).
28. Miller, J. A., Sandin, R. B., Miller, E. C., and Rush, H. P. The carcinogenicity of compounds related to 2-acetylaminofluorene. Cancer Res. 15: 188–199 (1955).
29. Schuetzle, D., Riley, T. L., Prater, T. J., Harvey, T. M., and Hunt, D. F. Analysis of nitrated polycyclic aromatic hydrocarbons in diesel particulate. Anal. Chem. 54: 265–271 (1982).
30. Henderson, T. R., Royer, R. E., Clark, C. R., Harvey, T. M., and Hunt, D. F. MS/MS analysis of diesel emissions and fuels treated with NO2. J. Appl. Toxicol. 2: 231–237 (1982).
31. Schuetzle, D. Sampling of vehicle emissions for chemical analysis and biological testing. Environ. Health Perspect. 47: 65–80 (1983).
32. Xu, X., Nachtman, J., Rappaport, S., and Wei, E. Identification of 2-nitrofluorene in diesel exhaust particulates. J. Appl. Toxicol. 1: 196–198 (1981).
33. Nishioka, M. G., Peterson, B., and Lewtas, J. Comparison of nitroaromatic content and direct-acting mutagenicity of passenger car engine emissions. In: Mobile Source Emissions Including Polycyclic Organic Species (D. Rondial, Ed.), D. Riedel Publishing Company, Dordrecht, Germany, 1983, p. 197.
34. Schuetzle, D., and Frazier, J. A. Factors influencing the emission of vapor and particulate phase components from diesel engines. In: Carcinogenic and Mutagenic Effects of Diesel Engine Exhaust (N. Ishinishi, A. Koizumi, R. O. McClellan, and W. Stöber, Eds.), Elsevier, Amsterdam, 1986, pp. 41–64.
35. IARC. Diesel and Gasoline Engine Exhausts and Some Nitroarenes. Monograph 46, International Agency for Research on Cancer, Lyon, 1989.
36. Möller, L., and Gustafsson, J.-Å. Liquid chromatographic mass spectrometric analysis of 2-nitrofluorene and its derivatives. Biomed. Mass. Spectrom. 13: 681–688 (1986).
37. Möller, L., Rafter, J., and Gustafsson, J.-Å. Metabolism of the carcinogenic air pollutant 2-nitrofluorene in the rat. Carcinogenesis 8: 637–645 (1987).
38. Möller, L., Törnquist, S., Beije, B., Rafter, J., Toftgård, R., and Gustafsson, J.-Å. Metabolism of the carcinogenic air pollutant 2-nitrofluorene in the isolated rat lung and liver. Carcinogenesis 8: 1847–1852 (1987).
39. Möller, L., Corrie, M., Midtvedt, T., Rafter, J., and Gustafsson, J.-Å. The role of the intestinal microflora in the formation of mutagenic metabolites from the carcinogenic air pollutant 2-nitrofluorene. Carcinogenesis 9: 823–830 (1988).
40. Törnquist, S., Möller, L., Gabrielsson, J., Gustafsson, J.-Å., and Toftgård, R. 2-Nitrofluorene metabolism in the rat lung. Pharmacokinetic and metabolic effects of β-naphthoflavone treatment. Carcinogenesis 11: 1249–1254 (1990).
41. Törnquist, S., Sundin, M., Möller, L., Gustafsson, J.-Å., and Toftgård, R. Age dependent expression of cytochrome P-450b and metabolism of the potent carcinogen 2-nitrofluorene in the rat lung. Carcinogenesis 9: 2209–2214 (1988).
42. Beije, B., and Möller, L. Unscheduled DNA synthesis in the liver and mutagenic activity in the urine of rats exposed to 2-nitrofluorene or 2-acetylaminofluorene. Environ. Mutagen. 8: 10 (1986).
43. Beije, B. and Möller, L. Correlation between induction of unscheduled DNA synthesis in the liver and excretion of mutagenic metabolites in the urine of rats exposed to the carcinogenic air pollutant 2-nitrofluorene. Carcinogenesis 9: 1465–1470 (1988).
44. Möller, L., Torndal, U.-B., Gustafsson, J.-Å., and Eriksson, L. C. The air pollutant 2-nitrofluorene as initiator and promotor in a liver model for studies on chemical carcinogenesis. Carcinogenesis 10: 435–440 (1989).
45. Beije, B., and Möller, L. 2-Nitrofluorene and related compounds. Prevalence and biological effects. Mutat. Res. 196: 177–209 (1988).
46. Rosenkranz, H. S., and Mermelstein, R. Mutagenicity and genotoxicity of nitroarenes. All nitro-containing chemicals were not created equal. Mutat. Res. 114: 217–267 (1983).
47. Wilson, R. H., DeEds, F., and Cox, A. J. The toxicity and carcinogenic activity of 2-acetylaminofluorene. Cancer Res. 1: 595–608 (1941).
48. Thorgeirsson, S. S., Weisburger, E. K., King, C. M., and Scribner, J. D. Carcinogenic and mutagenic N-substituted aryl compounds. NCI Monograph, No. 58, National Cancer Institute, Bethesda, MD, 1979, pp. 1–251, .
49. Campbell, R. M., and Lee, M. L. Capillary column gas chromatographic determination of nitro polycyclic aromatic compounds in particulate extracts. Anal. Chem. 56: 1026–1030 (1984).
50. Bertilsson, T., and Egebäck, K.-E. Emissions from diesel vehicles. Swedish EPA Report, SNV PM 1739, Stockholm, 1984, p. 19.
51. Schuetzle, D., and Perez, J. M. Factors influencing the emissions of nitrated-polynuclear aromatic hydrocarbons (nitro-PAH) from diesel engines. J. Air Pollut. Control Assoc. 33: 751–755 (1983).
52. Bond, J. A., Medinsky, M. A., and Dutcher, J. S. Metabolism of 1-[^{14}C]-nitropyrene in isolated perfused rat livers. Toxicol. Appl. Pharmacol. 75: 531–538 (1984).
53. Kinouchi, T., Morotomi, M., Mutai, M., Fifer, E. K., Beland, F. A., and Ohnishi, Y. Metabolism of 1-nitropyrene in germ-free and conventional rats. Jpn. J. Cancer Res. 77: 356–369 (1986).
54. Weisburger, E. K. N-substituted aryl compounds in carcinogenesis and mutagenesis. NCI Monograph, No. 58, National Cancer Institute, Bethesda, MD, 1979, p. 1.
55. McCann, J., Choi, E., Yamasaki, E., and Ames, B. Detection of carcinogens as mutagens in the salmonella/microsome test: assay of 300 chemicals. Proc. Natl. Acad. Sci. U.S.A. 72: 5135–5139 (1975).
56. Morris, H. P., Dubnik, C. S., and Johnson, J. M. Studies of the carcinogenic action in the rat of 2-nitro, 2-amino, 2-acetylamino, and 2-diacetylaminofluorene after ingestion and after painting. J. Natl. Cancer Inst. 10: 1201–1213 (1950).
57. Cerniglia, C. E., Howard, P. C., Fu, P. P., and Franklin, W. Metabolism of nitropolycyclic aromatic hydrocarbons by human intestinal microflora. Biochem. Biophys. Res. Commun. 123: 262–270 (1984).
58. Howard, P. C., Beland, F. A., and Carniglia, C. E. Reduction of the carcinogen 1-nitropyrene to 1-aminopyrene by rat intestinal bacteria. Carcinogenesis 4: 985–990 (1983).
59. El Bayoumi, K., Sharma, C., Louis, Y. M., Reddy, B., and Hecht, S. S. The role of intestinal microflora in the metabolic reduction of 1-nitropyrene to 1-aminopyrene in conventional and germfree rats and in humans. Cancer Lett. 19: 311–316 (1983).
60. Harries, G. C., Boobis, A. R., Coller, N., and Davies, D. S. Interindividual differences in the activation of two hepatic carcinogens to mutagens by human liver. Human Toxicol. 5: 21–26 (1986).
61. Eddy, E. P., Howard, P. C., McCoy, G. D., and Rosenkranz, H. S. Mutagenicity, unscheduled DNA synthesis, and metabolism of 1-nitropyrene in the human hepatoma cell line HepG2. Cancer Res. 47: 3163–3168 (1987).
62. Boobis, A. R., Brodie, M. J., McManus, M. E., Staiano, N., Thorgeirsson, S. S., and Davies, D. S. Metabolism and mutagenic activation of 2-acetylaminofluorene by human liver and lung. Adv. Exp. Med. Biol. 136: 1193–1201 (1981).
63. McManus, M. E., Trainor, K. J., Morley, A. A., Burgess, W., Stupans, I., and Birkett, D. J. Metabolism of 2-acetylaminofluorene in cultured human lymphocytes. Chem. Pathol. Pharmacol. 55: 409–418 (1987).
64. Moore, B. P., Hicks, R. M., Knowles, M. A., and Redgraves, S. Metabolism and binding of benzo[a]pyrene and 2-acetylaminofluorene by short-term organ cultures of human and rat bladder. Cancer Res. 42: 642–648 (1982).
65. Weisburger, J. H., Grantham, P. H., van Horn, E., Steigbigel, N. H., Rall, D. P., and Weisburger, E. K. Activation and detoxification of N-2-fluoreneylacetamide in man. Cancer Res. 24: 475–479 (1964).
66. Littlefield, N. A., Farmer, J. H., Gaylor, D. W., and Sheldon, W. G. Effects of dose and time in a long-term, low-dose carcinogenic study. J. Environ. Pathol. Toxicol. 3: 17–34 (1979).
67. Möller, J., Rafter, J., Törnquist, S., Eriksson, L., Beije, B., Toftgård, R., Midtvedt, T., Corrie, M., and Gustafsson, J.-Å. In vivo metabolism and genotoxic effects of the air pollutant and marker for nitro-PAHs, 2-nitrofluorene. Environ. Sci. Res. 40: 39–59 (1990).
68. ICRP. Recommendations of the International Commission on Radiological Protection, Publication No. 26, Vol. 1, No. 3. Pergamon Press, Oxford, 1977.

69. Massaro, M., McCartney, M., Rosenkranz, E. J., Anders, M., McCoy, E., Mermelstein, R., and Rosenkranz, H. S. Evidence that nitroarene metabolites form mutagenic adducts with DNA-adenine as well as with DNA-guanine. Mutat. Res. 122: 243–249 (1983).
70. Suzuki, Y. Studies on development of the sensitive micronucleus test. Part 2. The in vitro method using cultured bone marrow cells. Tokyo Jikeikai Med. J. 100: 707–719 (1985).
71. Sakitani, T. and Suzuki, Y. Part 2. Mutagenic activities of air pollutants observed by micronucleus test. Tokyo Jikeikai Med. J. 101: 259–266 (1986).
72. Ohe, T. Studies on comparative decomposition rate by liver homogenate an on micronucleus test of nitrated polycyclic aromatic hydrocarbons. Bull. Environ. Contam. Toxicol. 34: 715–721 (1985).
73. Möller, L., Lax, I., Torndal, V.-B., and Erikson, L. C. Risk assessment of nitrated polycyclic aromatic hydrocarbons. Risk Analysis, in press.
74. Rosenkranz, H. S., McCoy, E. C., Frierson, M., and Klopman, G. The role of DNA sequence and structure of the electrophile on the mutagenicity. Environ. Mutagen. 7: 645–653 (1985).
75. Andrews, L. S., Pohl, L. R., Hinson, J. A., and Gilette, J. R. Mutagenesis of 2-nitrofluorene (NF), 2-nitrosofluorene (NOF) and 2-hydroxylaminofluorene (NHOHF) for Salmonella TA 100 and TA 200 FR. Toxicol. Appl. Pharmacol. 48: A48 (1979).
76. Banerjee, T. S., Bhaumik, G., Yu, C.-L., Swaminathan, B., Giri, A. K., Srivastava, S., and Bhattacharjee, S. B. Evaluation of the genotoxicity of lac dye. Food Chem. Toxicol. 22: 677–679 (1984).
77. Doudney, C. O., Franke, M. A., and Rinaldi, C. N. The DNA damage activity (DDA) assay and its application to river water and diesel exhausts. Environ. Int. 5: 293–297 (1981).
78. Wangenheim, J., and Bolcsfoldi, G. Mouse lymphoma TK +/− assay of 30 compounds. Environ. Mutagen. 8: 90 (1986).
79. Iida, Y., Daishima, S., Furuya, K., Kikuchi, T., Matsushita, H., Tanabe, K., Wu, J., Wan, A.-P., and Huang, Y.-C. Japan-China cooperative study: present state of air pollution in Beijing. Sekeij; Asian Pacific Studies 111–129 (1987).
80. Moriske, H. J., Block, I., and Rüden, H. Zur Mutagenen Wirksamkeit Polarer Organischer Verbindungen in Luftsäuben eines mit Hausbrand und eines mit Kfz-Verkehr Belasteten Messtandortes. In: Scritten Reihe Verein Wasser-Boden-Lufthygiene 67. Gustav Fischer Verlag, Stuttgart, 1986, p. 235.
81. Robert, L. Atomic bomb doses reassessed. Science 238: 1649–1651 (1987).
82. Faustman-Watts, E. M., Greenaway, J. C., Namkung, M. J., Fantel, A. G., and Juchau, M. R. Teratogenicity in vitro of two deacetylated metabolites of N-hydroxy-2-acetylaminofluorene. Toxicol. Appl. Pharmacol. 76: 161–171 (1984).
83. Moon, R. C., Rao, K. V. N., and Detrisac, C. J. Potential carcinogenicity of 1-nitropyrene. In: The Fifth Health Effects Institute Annual Conference, Health Effects Institute, Colorado Springs, CO, 1988, p. 15.
84. Moriske, H.-J., Block, I., Schleibinger, H., and Rüden, H. Polar neutral organic compounds in urban aerosols. Zbl. Bakt. Hyg. 181: 240–271 (1985).